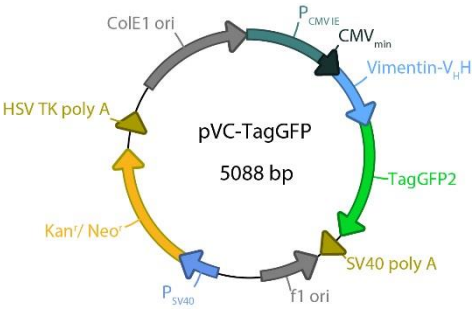
Vimentin Chromobody®-TagGFP plasmid

The plasmid map has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by ChromoTek. This vector has not been completely sequenced.
For plasmid sequence, please contact info@chromotek.com



Product	Code	Size
pVC-TagGFP	vcg	20 µg plasmid DNA (1 g/L)

Vector type	mammalian expression vector
Reporter	TagGFP2
Reporter codon usage	mammalian
Promoter for Chromobody®	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic – kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Vimentin Chromobody®-TagGFP expression in mammalian cells for live-cell visualization of endogenous vimentin filaments

Location of features

PCMV IE: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

Vimentin-V_HH (VB6): 621-977

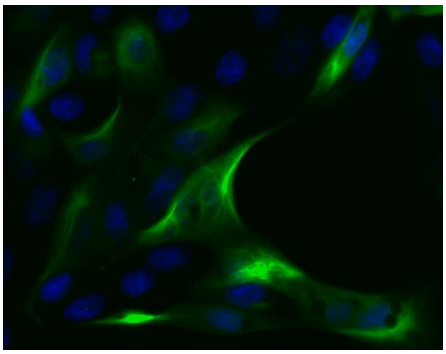
TagGFP2: 1038-1754

Stop codon: 1752-1754

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2984-2986; Stop codon: 3776-3778



MDCK cells transfected with pVC-TagGFP2 plasmid. The cells were fixed 48 h after transfection, cell nuclei were stained with DAPI (in blue). Vimentin-V_HH-TagGFP highlights vimentin intermediate filaments in transfected cells (in green).

Vector description

Vimentin Chromobody®-TagGFP plasmid (pVC-TagGFP) is a mammalian expression vector encoding the marker of vimentin intermediate filaments Vimentin-V_HH fused to the green fluorescent protein TagGFP2 (evrogen.com). The vector allows expression Vimentin Chromobody®-TagGFP fusion protein in eukaryotic (mammalian) cells. Chromobody® codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996].

The vector backbone contains immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, ColE1 origin of replication for propagation in *E. coli* and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3'-end of the reporter mRNA.

SV40 early promoter (P_{SV40}) provides neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan^r) in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Expression in mammalian cells

pVC-TagGFP vector can be transfected into mammalian cells by any known transfection method, e.g. with Lipofectamine® 2000 according to the manufacturer's instructions (thermofisher.com). If required, stable transformants can be selected using G418 [Gorman 1985].

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

Note: The plasmid DNA was isolated from dam^+-methylated *E.coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.